Folate deficiency enhances arsenic effects on expression of genes involved in epidermal differentiation.

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US Environmental Protection Agency

US EPA Office of Research and Development

Objective

The objective is to improve understanding of the molecular mechanisms of arsenic-induced skin cancer, and the influence of dietary folate deficiency on this process. For these studies, arsenic-induced changes in global gene expression are evaluated in skin of K6/ODC transgenic mice maintained on folate-sufficient or folate-deficient diets.

Background

Exposure to arsenic in the environment presents a widespread cancer hazard. Recent studies have identified low dietary folic acid (folate) as one of the nutritional susceptibility factors for arsenic-induced non-neoplastic skin lesions which are known to be developmentally associated with skin cancers. Folate is a B vitamin that normally functions in DNA methylation, synthesis and repair. Studies in rodent and human cells have shown that folate deficiency can enhance the toxicities of arsenic and other chemicals. This study evaluates the combined effects of arsenic and folate deficiency on gene expression in the skin of K6/ODC transgenic mice, a rodent model in which arsenic treatment alone has been shown to be tumorigenic (Chen *et al.*, 2000, 2002). These mice overexpress ornithine decarboxylase (ODC) in the hair follicle keratinocytes, the probable target cells for skin tumorigenesis. Overexpression of ODC, an enzyme in the polyamine biosynthetic pathway and a biomarker of epithelial cell proliferation, is widely believed to contribute to skin tumorigenesis in rodents and humans.

Methods

Mice: K6/ODC female mice from Taconic Farms, Inc.

Diet: Folate-deficient purified diet with vitamin free casein, corn oil and 1% succinylsulfathiazole (eliminates contribution of the intestinal microflora to folate availability), or the same diet supplemented with 5 mg/kg folic acid (Dyets, Inc.)

Treatment 0, 1, or 10 ppm sodium arsenite in the drinking water for 30 days. 24 samples, 4 animals per each of 6 treatment conditions (3 arsenic doses, each with folate- deficient and sufficient diets)

Serum folate and total homocysteine: Bayer Diagnostics ADVIA Centaur Automated Chemiluminescense system and AxSYM Immunoassay Analyzer (Abbot Laboratories), respectively

Skin samples: harvested from mid-back

Total RNA: TriReagent procedure (Molecular Research Center) and Qiagen RNeasy mini RNA cleanup protocol

Gene expression: Affymetrix 430 2.0 genechips

Data Analysis: Genespring GX 7.3 Expression Analysis Software, Ingenuity Pathways Analysis Software, and oPOSSUM 1.3

Results

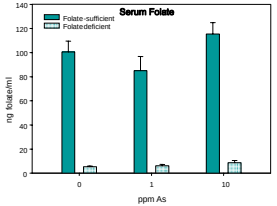


Fig. 1. Serum folate and total blood homocysteine levels. Bars represent the mean and standard error of 4 samples per treatment.

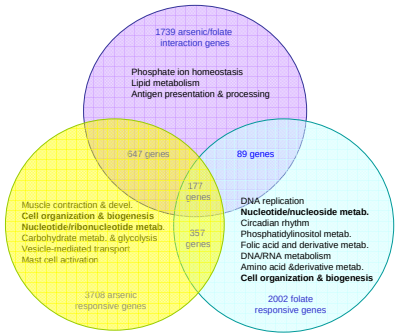


Fig. 2. Venn diagram of Two-way ANOVA ($p \leq 0.05$) results. Significant GO categories ($p \leq 0.001$) are listed for each parameter in order of significance.

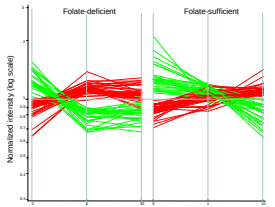
Table 1. Functional analysis of arsenic-responsive genes using Ingenuity Pathways Analysis.

Folate-sufficient, 1 ppm As
Tissue Morphology
Skeletal & Muscular System Development & Function
Immune Response
Cell-to-Cell Signaling and Interaction
Hematological System Development & Function
Immune & Lymphatic System Development & Function
Tissue Development
Carbohydrate Metabolism
Molecular Transport
Small Molecule Biochemistry

Folate-deficient, 1 ppm As
Tissue Morphology
Skeletal & Muscular System Development & Function
Hair & Skin Development & Function
Organ Development
Cellular Compromise
Immune Response
Immune & Lymphatic System Development & Function
Nucleic Acid Metabolism
Hematological Disease
Respiratory Disease

Folate-deficient, 10 ppm As
Hair & Skin Development & Function
Organ Development
Immune Response
Cellular Movement
Hematological System Development & Function
Free Radical Scavenging
Tissue Morphology
Cellular Growth and Proliferation
Skeletal & Muscular System Development & Function
Organismal Injury and Abnormalities

Folate-deficient, 10 ppm As
Viral Function
Hair & Skin Development & Function
Organ Development
Cellular Development
Hematological System Development & Function
Behavior
Cellular Growth and Proliferation
Skeletal & Muscular System Development & Function
Renal & Urological Disease
Amino Acid Metabolism



Gene Symbol

1110055L24Rik, Adam12, Adam12, Aoc3, Aspn, Bgn, Bgn, Cd209a, Cd36, Cd36, Cd97, Col14a1, Col4a1, Col5a2, Col8a2, Cspg2, Cspg2, Cyr61*, Cyr61, Dcn, Dpt, Ednrb*, Fbln5, Fbn1, Fn1, Itgb1, Itgb1, Itgb1*, Kit *, Klrb1b, Lama2, Lum, Mfap5 *, Mfap5, Mgl2, Mpdz, Omd, Pcdh18, Postn, Prelp, Reg1, Sparcl1, Timp3 *, Timp3 *, Tm4sf1*, Tm4sf6, Involucrin*, Alox12b*, Casp14*, Cdkn1a, Col17a1*, Dlx3*, Evpl, Flg*, Gjb5*, Hr*, Itga3, Krt1-10*, Krt1-23*, Krt2-1*, Krt2-17, Lor*, Lor*, Mt4*, Notch1, Sbsn*, Sprr1b*, Sprr2h, Sprr2f*, Sprr3*, Sprr3*, Sprr5*, Sprr7*, Tgm1

Fig. 3. Expression profiles and associated gene lists for extracellular matrix (ECM) and epidermal differentiation genes significantly ($p \leq 0.01$) altered by arsenic treatment (red=upregulated; green=downregulated). An * denotes the gene is also significantly altered by folate deficiency at the 1 ppm As dose ($p \leq 0.05$, in the same direction as the As response).

Table 2. Transcription factor binding site analysis results with oPOSSUM™

Transcription factor	Class	Target gene hits	Fisher P-value	Z-score
Arsenic responsive genes (3708)				
Staf	ZN-FINGER, C2H2	128	4.03E-03	13.17
CREB	bZIP	425	1.90E-02	5.85
NRF-2	ETS	433	2.40E-02	5.87
SRF	MADS	70	3.11E-02	7.18
p65	REL	402	4.77E-02	5.85
Epidermal differentiation and extracellular matrix gene subset (65)				
c-FOS	bZIP	30	7.55E-05	7.33
FREAC-2	FORKHEAD	15	1.91E-04	7.64
SRF	MADS	6	1.28E-03	11.22
HNF-3beta	FORKHEAD	25	1.35E-03	6.80
HFH-1	FORKHEAD	18	2.17E-03	5.44
E2F	UNKNOWN	13	4.20E-03	6.95
Thing1-E47	bHLH	28	7.44E-03	8.81
Androgen	NUCLEAR RECEPTOR	3	4.73E-02	6.78

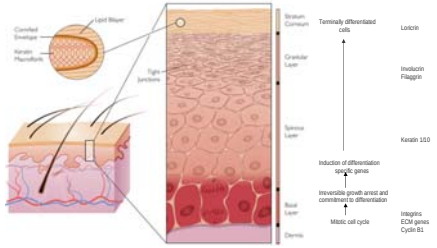
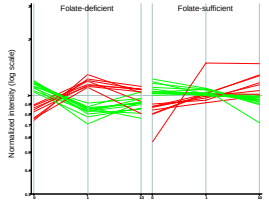


Fig. 4. Schematic diagram of the stages of epidermal differentiation (J. Segre, NHGRI, www.genome.gov)



Gene Symbol

Pdgfra, Fgf7, Fos, Eps8, Tgfbr2, Fgf7, Pdgfc *, Ccnb1*, Tgfb3 *, Notch1, Cebpa, Ogfr *, Flbp, Hras1*, Cdkn1a, Grb7 *, Tbrg4, Vegfb *, Krt1-10 *, Nrtn *

Fig. 5. Expression profiles and gene list for growth and proliferation-related genes significantly ($p \leq 0.01$) altered by arsenic treatement (red=upregulated; green=downregulated). An * denotes the gene is also significantly altered by folate deficiency at the 1 ppm As dose ($p \leq 0.05$, in the same direction as As response).

Summary

- Mice maintained on the folate deficient diet had lower serum folate levels and increased homocysteine levels (Fig. 1).
- Both arsenic treatment and folate deficiency, separately and together, altered the expression of genes in categories of nucleotide metabolism, and cell organization and biogenesis (Fig. 2). The latter category includes genes functioning in intracellular protein transport, microtubule-based processes, cytoskeleton organization and epidermal differentiation.
- Genes involved in hair and skin development/function serve as markers of epidermal differentiation, and are down regulated by arsenic (Table 1, Fig 3). At the lowest arsenic dose of 1 ppm, these genes are suppressed for animals on the folate-deficient, but not the folate-sufficient, diet.
- Many extracellular matrix (ECM) genes which help regulate epidermal differentiation were upregulated by arsenic (Fig. 3). For some of these genes which have been implicated in progression of skin cancers, e.g., Cyr 61, TM4SF1 and TIMP3, this effect was seen at a lower arsenic dose in animals maintained on the folate-deficient diet as compared to those on the folate-sufficient diet.
- Arsenic increased the expression of several growth factor genes and decreased the expression of several genes functioning in negative growth regulation, e.g., cdkn1a (p21), cebpa, krt1-10, and notch 1 (Fig. 5). For some of these genes, the arsenic effective dose was lower under the condition of folate deficiency.
- Staf, a zinc-finger transcription factor, and CREB, a bZIP transcription factor, were the most significantly over-represented transcription factors for the arsenic responsive genes (Table 2). For the combined ECM and epidermal differentiation gene lists, c-FOS is the most significant transcription factor binding site, suggesting regulation of these genes by AP-1.

Conclusion

Our gene profiling results suggest that the normal balance between epidermal proliferation and differentiation is disrupted by arsenic ingestion, and that folate deficiency lowers the effective dose for this effect. Arsenic causes suppressed expression of key marker genes for keratinocyte differentiation and increased expression of ECM genes, both effects being greater at the low dose (1 ppm) under conditions of folate deficiency. These findings support the view that dietary folate deficiency may impact arsenic response genes to enhance carcinogenic effects in skin.